

Attorney Docket No.: 44158/244344 (SJ-0029)
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This listing of the claims will replace all prior versions and listings of claims in the application:

Listing of the claims:

Claim 1: (currently amended) A method for predicting the ~~level and distribution of~~ CYP3A5 expression level in a subject comprising determining the nucleotide present in each CYP3A5 allele of the genomic DNA of said subject at the location(s) selected from the group consisting of:

(a) the position corresponding to nucleotide ~~22,893 of~~ ~~Genbank sequence accession no. AC005020~~ 23 of SEO ID NO:73 within intron 3 of the Cyp3A5 gene:

(b) the position corresponding to nucleotide ~~30,597 of~~ ~~Genbank sequence accession no. AC005020~~ 29 of SEO ID NO:74 within exon 7 of the Cyp3A5 gene; and

(c) the positions corresponding to both nucleotide ~~22,893~~ and nucleotide ~~30,597 of Genbank sequence accession no. AC005020~~ 23 of SEO ID NO:73 and nucleotide 29 of SEO ID NO:74;

wherein the presence of an A at the position corresponding to nucleotide ~~22,893 of Genbank sequence accession no. AC005020~~ 23 of SEO ID NO:73 on at least one CYP3A5 allele of said subject predicts a relatively high level of expression of CYP3A5 and the presence of a G at the position corresponding to nucleotide

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~~22,893 of Genbank sequence accession no. AC005020~~ 23 of SEQ ID NO:73 on each CYP3A5 allele of said subject predicts a relatively low level of expression;

wherein the presence of a G at the position corresponding to nucleotide ~~30,597 of Genbank sequence accession no. AC005020~~ 29 of SEQ ID NO:74 on at least one CYP3A5 allele of said subject predicts a relatively high level of expression of CYP3A5 and the presence of an A at the position corresponding to nucleotide ~~30,597 of Genbank sequence accession no. AC005020~~ 29 of SEQ ID NO:74 on each CYP3A5 allele of said subject predicts a relatively low level of expression of CYP3A5; and

wherein the presence of an A at the position corresponding to nucleotide ~~22,893 of Genbank sequence accession no. AC005020~~ 23 of SEQ ID NO:73 and a G at the position corresponding to nucleotide ~~30,597 of Genbank sequence accession no. AC005020~~ 29 of SEQ ID NO:74 on at least one CYP3A5 allele of said subject predicts a relatively high level of expression of CYP3A5 and the presence of either a G at the position corresponding to nucleotide ~~22,893 of Genbank sequence accession no. AC005020~~ 23 of SEQ ID NO:73 or an A at the position corresponding to nucleotide ~~30,597 of Genbank sequence accession no. AC005020~~ 29 of SEQ ID NO:74 on each CYP3A5 allele of said subject predicts a

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relatively low level of expression of CYP3A5.

Claim 2: (currently amended) The method of claim 1 wherein said location is the position corresponding to nucleotide ~~22,893~~ of ~~Genbank sequence accession no. AC005020~~ 23 of SEQ ID NO:73 within intron 3 of the Cyp3A5 gene.

Claim 3: (currently amended) The method of claim 1 wherein said location is the position corresponding to nucleotide ~~30,597~~ of ~~Genbank sequence accession no. AC005020~~ exon 5 29 of SEQ ID NO:74 within exon 7 of the Cyp3A5 gene.

Claim 4: (currently amended) The method of claim 1 wherein said locations are the positions corresponding to both nucleotide ~~22,893~~ and nucleotide ~~30597~~ of ~~Genbank sequence accession no. AC005020~~ 23 of SEQ ID NO:73 and nucleotide 29 of SEQ ID NO:74.

Claim 5: (currently amended) The method of claims ~~1-4~~ 1, 2, 3 or 4 wherein the step of determining the nucleotide present in each CYP3A5 allele of said subject at the selected location(s) is accomplished by sequencing a region of the genomic DNA of said subject which includes said location(s).

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Claim 6: (currently amended) The method of claims ~~1-4~~ 1, 2, 3 or 4 wherein the step of determining the nucleotide present in each Cyp3A5 allele of said subject at the selected location(s) is accomplished by

(a) amplifying a region of the genomic DNA of said subject which includes said location(s) to generate an amplified fragment, and

(b) treating the amplified fragment with a restriction enzyme in its corresponding restriction buffer to determine the identity of the nucleotide present at the selected location(s).

Claim 7: (currently amended) The method of claims ~~1-4~~ 1, 2, 3 or 4 wherein the step of determining the nucleotide present in each Cyp3A5 allele of said subject at the selected location(s) is accomplished by

(a) amplifying a region of the genomic DNA of said subject which includes said location(s), and

(b) hybridizing the amplified region with probes specific for the selected location(s) wherein hybridization determines the identity of the nucleotide present at the selected location(s).

Claim 8: (currently amended) A method for determining the

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cytochrome P450 3A5 (CYP3A5) genotype and phenotype of an individual comprising:

- (a) isolating nucleic acid from the individual;
- (b) amplifying a region of the cytochrome P450 3A5 (CYP3A5) gene sequence selected from the group of:

- (i) intron 3 comprising the position corresponding to nucleotide 22,893 of Genbank accession no. AC005020 23 of SEQ ID NO:73;

- (ii) exon 7 comprising the position corresponding to nucleotide 30,597 of Genbank accession no. AC005020 29 of SEQ ID NO:74; and

- (iii) intron 3 comprising the position corresponding to nucleotide 22,893 of Genbank accession no. AC005020 23 of SEQ ID NO:73 and exon 7 comprising the position corresponding to nucleotide 30,597 of Genbank accession no. AC005020 29 of SEQ ID NO:74; and

- (c) analyzing the cytochrome P450 3A5 (CYP3A5) sequence sequencing the amplified region of step (b), thereby determining the cytochrome P450 3A5 (CYP3A5) genotype and phenotype of the individual.

Claim 9: (currently amended) The method of claim 8 wherein

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the intron 3 region of cytochrome P450 3A5 (CYP3A5) is amplified utilizing primers which amplify 5' and 3' of the nucleotide ~~22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73.

Claim 10: (currently amended) The method of claim 9 wherein the intron 3 region is amplified utilizing ~~primer pairs~~ SEQ ID NO: 24 and 25 primers, or ~~primer pairs~~ SEQ ID NO: 26 and 27 primers.

Claim 11: (currently amended) The method of claim 8 wherein the exon 7 region of cytochrome P450 3A5 (CYP3A5) is amplified utilizing primers which amplify 5' and 3' of the nucleotide ~~30,597 point mutation of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74.

Claim 12: (currently amended) The method of claim 11 wherein the exon 7 region is amplified utilizing ~~primer pairs~~ SEQ ID NO: 30 and 16 primers, or ~~primer pairs~~ SEQ ID NO: 31 and 32 primers.

Claim 13: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) intron 3 genotype of a subject which

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comprises:

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a set of primers, wherein said set of primers contains primer X and primer Y; wherein
 - (i) the X primer is complementary to a region 5' to the ~~point mutation site at nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73; and
 - ~~(iii)~~ (ii) the Y primer is complementary to a region 3' to the ~~point mutation site at nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73;
- ~~(c) amplifying and the sequence cytochrome P450 3A5 (CYP3A5) PCR fragment amplified is in between primers X and Y, thereby obtaining an amplified fragment; and~~
- ~~(d)~~ (c) sequencing the amplified fragment obtained in step ~~(c)~~ (b), thereby determining the cytochrome P450 3A5 (CYP3A5) intron 3 genotype of said subject.

Claim 14: (original) The method of claim 13 wherein primer X has the sequence corresponding to SEQ ID NO: 24, or a fragment

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thereof which is at least ten bases long, and primer Y has the sequence corresponding to SEQ ID NO: 25, or a fragment thereof which is at least ten bases long.

Claim 15: (original) The method of claim 13 wherein primer X has the sequence corresponding to SEQ ID NO: 26, or a fragment thereof which is at least ten bases long, and primer Y has the sequence corresponding to SEQ ID NO: 27, or a fragment thereof which is at least ten bases long.

Claim 16: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises:

- (a) isolating nucleic acid from said subject;
- (b) making a first and a second PCR primer wherein
 - (i) the first PCR primer is complementary to intron 3 and introduces a base change in the PCR product adjacent to or near the ~~point mutation at nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73, such that a restriction site is generated in the presence of a particular nucleotide at the position corresponding to nucleotide 22,893 23 of SEQ ID NO:73 in the PCR product; and

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(ii) the second PCR primer is complementary to a region 3' to the ~~intron 3 nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73 of intron 3;

(c) amplifying the sequence in between the first and the second primers; thereby obtaining an amplified fragment; and

(d) treating the amplified fragment obtained in step (c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at ~~nucleotide 22,893 of Genbank accession no. AC005020~~ the position corresponding to nucleotide 23 of SEQ ID NO:73, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Claim 17: (currently amended) The method of claim 16 wherein the first primer introduces a *Tru9I/MseI* restriction site in the presence of an A nucleotide at ~~nucleotide 22,893~~ the position corresponding to nucleotide 23 of SEQ ID NO:73, and the second primer has the sequence selected from SEQ ID NO:27 and SEQ ID NO: 25, or a fragment thereof which is at least ten bases long.

Claim 18: (original) The method of claim 16 wherein the

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first primer has the sequence corresponding to SEQ ID NO: 33, or a fragment thereof which is at least ten bases long, and the second primer has the sequence corresponding to SEQ ID NO: 27, or a fragment thereof which is at least ten bases long.

Claim 19: (original) The method of claim 16 wherein the first primer has the sequence corresponding to SEQ ID NO:33 , or a fragment thereof which is at least ten bases long, and the second primer has the sequence corresponding to SEQ ID NO:25, or a fragment thereof which is at least ten bases long.

Claim 20: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises:

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a first set of primers, wherein said first set of primers contains primer X and primer Y; wherein
 - (i) the X primer is complementary to a region 5' to the ~~point mutation site at nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73;
and

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(ii) the Y primer is complementary to a region 3' to the ~~point mutation site at nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73;

~~(c) amplifying and the sequence cytochrome P450 3A5 (CYP3A5) PCR fragment amplified is in between primers X and Y, thereby obtaining an first round amplified fragment;~~

~~(d)~~ (c) amplifying the first round amplified fragment of step (b) using a second set of primers, wherein said second set of primers contains primer Z and primer W, wherein

(i) primer Z is complementary to intron 3 and introduces a base change in the PCR product adjacent to or near the ~~point mutation at nucleotide 22,893 of Genbank accession no. AC005020~~ position corresponding to nucleotide 23 of SEQ ID NO:73, such that a restriction site is generated in the presence of a particular mutation at ~~nucleotide 22,893~~ the position corresponding to nucleotide 23 of SEQ ID NO:73; and

(ii) primer W is complementary to a region 3' to intron 3;

~~(e) amplifying and the amplified sequence is in between primers Z and W, thereby obtaining an amplified fragment; and~~

~~(f)~~ (d) treating the amplified fragment obtained in step ~~(e)~~

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(c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at ~~nucleotide 22,893 of Genbank accession no. AC005020~~ the position corresponding to nucleotide 23 of SEQ ID NO:73, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Claim 21: (currently amended) The method of claim 20 wherein primer X has the sequence corresponding to SEQ ID NO: 24, or a fragment thereof which is at least ten bases long; primer Y has the sequence selected from the group of SEQ ID NO:25, or a fragment thereof which is at least ten bases long; primer Z introduces a *Tru9I/MseI* restriction site in the presence of an A nucleotide at ~~nucleotide 22,893 of Genbank accession no. AC005020~~ the position corresponding to nucleotide 23 of SEQ ID NO:73; and primer W has the sequence selected from SEQ ID NO: 27 and SEQ ID NO: 25, or a fragment thereof which is at least ten bases long.

Claim 22: (original) The method of claim 21 wherein primer Z has the sequence corresponding to SEQ ID NO: 33, or a fragment thereof which is at least ten bases long.

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Claim 23: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises

(a) isolating nucleic acid from said subject;
(b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a set of primers, wherein said set of primers contains primer X and primer Y; wherein

(i) the X primer is complementary to a region 5' to the ~~point mutation site at nucleotide 30,597 of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74;

~~(iii)~~ (ii) the Y primer is complementary to a region 3' to the ~~point mutation site at nucleotide 30,597 of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74;

~~(c) amplifying and the sequence amplified cytochrome P450 3A5 (CYP3A5) PCR fragment is in between primers X and Y, thereby obtaining an amplified fragment; and~~

~~(d)~~ (c) sequencing the amplified fragment obtained in step ~~(c)~~ (b), thereby determining the cytochrome P450 3A5 (CYP3A5) exon 7 genotype of said subject.

Claim 24: (original) The method of claim 23 wherein primer X

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has the sequence corresponding to SEQ ID NO: 30, or a fragment thereof which is at least ten bases long, and primer Y 20 has the sequence corresponding to SEQ ID NO: 16, or a fragment thereof which is a least ten bases long.

Claim 25: (original) The method of claim 23 wherein primer X has the sequence corresponding to SEQ ID NO:31, or a fragment thereof which is at least ten bases long, and primer Y 25 has the sequence corresponding to SEQ ID NO:32 or a fragment thereof which is at least ten bases long.

Claim 26: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises:

- (a) isolating nucleic acid from said subject;
- (b) making a first and a second PCR primer wherein
 - (i) the first PCR primer is complementary to exon 7 and introduces a base change in the PCR product adjacent to or near the ~~point mutation at nucleotide 30,597 of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74, such that a restriction site is generated in the presence of a particular nucleotide at ~~nucleotide 30,597~~ the position

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corresponding to nucleotide 29 of SEQ ID NO:74; and

(ii) the second PCR primer is complementary to a region 3' to the ~~intron 3~~ exon 7 nucleotide ~~30,597 of Genbank accession no. AC005020~~ in the position corresponding to nucleotide 29 of SEQ ID NO:74;

(c) amplifying the sequence in between the first and the second primers; thereby obtaining an amplified fragment; and

(d) treating the amplified fragment obtained in step (c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at nucleotide ~~30,597 of Genbank accession no. AC005020~~ the position corresponding to nucleotide 29 of SEQ ID NO:74, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Claim 27: (currently amended) The method of claim 26 wherein the first primer introduces a *Tru9I/MseI* restriction site in the presence of ~~a an~~ A nucleotide at nucleotide ~~30,597 of Genbank accession no. AC005020~~ the position corresponding to nucleotide 29 of SEQ ID NO:74, and the second primer has the sequence selected from SEQ ID NO:32 and SEQ ID NO:16, or a fragment thereof which is at least ten bases long.

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Claim 28: (original) The method of claim 26 wherein the first primer has the sequence corresponding to SEQ ID NO: 34, or a fragment thereof which is at least ten bases long, and the second primer has the sequence corresponding to SEQ ID NO: 32, or a fragment thereof which is at least ten bases long.

Claim 29 (original) The method of claim 26 wherein the first primer has the sequence corresponding to SEQ ID NO:34, or a fragment thereof which is at least ten bases long, and second primer has the sequence corresponding to SEQ ID NO:16, or a fragment thereof which is at least ten bases long.

Claim 30: (currently amended) A method for determining cytochrome P450 3A5 (CYP3A5) exon 7 genotype of a subject which comprises:

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a first set of primers, wherein said first set of primers contains primer X and primer Y; wherein
 - (i) the X primer is complementary to a region 5' to the ~~point mutation site at nucleotide 30,597 of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74;

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and

(ii) the Y primer is complementary to a region 3' to the ~~point mutation site at nucleotide 30,597 of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74;

~~(c) amplifying and the sequence amplified cytochrome P450 3A5 (CYP3A5) PCR fragment is in between primers X and Y, thereby obtaining an a first round amplified fragment;~~

~~(d) (c) amplifying the first round amplified fragment of step (b) using a second set of primers, wherein said second set of primers contains primer Z and primer W, wherein~~

(i) primer Z is complementary to exon 7 and introduces a base change in the a PCR product adjacent to or near the ~~point mutation at nucleotide 30,597 of Genbank accession no. AC005020~~ position corresponding to nucleotide 29 of SEQ ID NO:74, such that a restriction site is generated in the presence of a particular mutation at ~~nucleotide 30,597 of Genbank accession no. AC005020~~ the position corresponding to nucleotide 29 of SEQ ID NO:74; and

(ii) primer W is complementary to a region 3' to exon 7;

~~(c) amplifying and the amplified sequence is in between~~

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primers Z and W, ~~thereby obtaining an amplified fragment; and~~
~~(f)~~ (d) treating the amplified fragment obtained in step ~~(e)~~
(c) with a restriction enzyme in its corresponding restriction
buffer to detect presence or absence of a point mutation at
~~nucleotide 30,597 of Genbank accession no. AC005020~~ the position
corresponding to nucleotide 29 of SEQ ID NO:74, thereby
determining the cytochrome P450 3A5 (CYP3A5) genotype of said
subject.

Claim 31: (currently amended) The method of claim 30 wherein
primer X has the sequence corresponding to SEQ ID NO:30, or a
fragment thereof which is at least ten bases long; primer Y has
the sequence of SEQ ID NO: 16, or a fragment thereof which is at
least ten bases long; primer Z introduces a Tru9I/MseI
restriction site in the presence of an A nucleotide at ~~nucleotide~~
~~30,597~~, the position corresponding to nucleotide 29 of SEQ ID
NO:74; and primer W has the sequence selected from SEQ ID NO:32
and SEQ ID NO:16, or a fragment thereof which is at least ten
bases long.

Claim 32: (original) The method of claim 31 wherein primer Z
has the sequence corresponding to SEQ ID NO:34, or a fragment

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thereof which is at least ten bases long.

Claim 33-38: (canceled)